

**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.**

An injection device injects small amounts of injectate into each of a plurality of cells (20). A first plate (12), which may be made from a silicon chip, includes a large number of evenly spaced cell wells (14), in a regular array. Each well includes a through hole (16) in the chip. A top plate (22), also formed from a silicon chip, includes a plurality of injection needles (24), arranged in a regular array corresponding to the position of the cell wells (14). A suspension of cells is washed over the first plate (12) and individual cells (20) are retained by the individual wells (14). A vacuum may be applied to the cell wells (14) through a manifold (32) beneath the first plate (12), thus to retain the cells (20) in place. Excess cells (20) and suspension are removed by washing the plate (12). The second plate (22), including the injection needles (24), is positioned on the first plate (12) so that the needles (24) pierce the plurality of cells (20) retained in the wells (14), thus simultaneously introducing the injectate into a large number of individual cells (20).

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## ARRAY-TYPE MULTIPLE CELL INJECTOR

Technical Field

This invention relates to injection devices, and more particularly to structure for substantially simultaneous injection of a medium into a plurality of individual cells.

Background Art

In modern biotechnological operations, it is often necessary to inject small amounts of protein, DNA, RNA drugs or other substances into each of a plurality of living cells. For example, quantities such as 20 to 50 nanoliters may be desired to be injected into each of a plurality of frog oocytes. Alternatively, a few nanoliters (1 to 10) may be required to be injected in mouse stem cells. These injections are frequently required to be made quickly and easily into large numbers of cells, for example, into thousands or tens of thousands of cells.

Such injection is presently carried out manually, utilizing micromanipulators to hold micropipettes, and further utilizing a microscope for visual observation. Some mechanization has been developed for such techniques. One device is available under the designation Stoelting Model 567-57-RH. Another is available under the designation Narishigi Model M0203. However, even with such devices, significant manual intervention is required. For example, in one known device, the cells are plated on a cover slip or petri dish. A computer controlled microscope having a TV camera is manipulated by an operator. A joy stick is used to manipulate the device until a cell is observed under a cross hair. The computer is given information to

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execute an injecti n program. However, such devices are expensive, do not create regular distributions of cells, operate slowly, and can only inject approximately 2,000 cells per hour. Thus, such devices cannot easily and  
5 inexpensively perform injections of tens of thousands of cells.

Moreover, such prior art devices do not work for cells in a suspension but are limited only to cell types which adhere to surfaces. Moreover, requirement of a  
10 human operator to identify the location of each of the cells to be injected adds yet a further expense to operation of the devices.

Electroporation is another known technology for introducing molecules into cells. This technique suffers  
15 from at least two disadvantages relative to the present invention, however. First, in typical electroporation, the substance to be introduced must be dispersed throughout the suspension medium, thus requiring the use of much more injectant material. Second, electroporation  
20 does not introduce material into the nucleus of the cell.

Lyposomes or calcium phosphate precipitation can also be used to deliver certain materials into cells. However, their use is somewhat limited.

Accordingly, there is a need in the prior art for  
25 a device which enables a more rapid injection of large numbers of cells, which does not require tedious human intervention, which is inexpensive, which may be disposable, which is highly efficient in the use of injecting solution which may be quite precious, which  
30 introduces injected material into the cell nucleus, which does not require the use of any special biological, which can deliver practically any water-soluble material into

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the cells and is parsimonious in its use of the valuable reagents, and which is constructed entirely of inert, biocompatible materials.

Disclosure of the Invention

5 It is accordingly an object of the present invention to overcome the deficiencies of the prior art and to provide apparatus for rapid injection of a medium into a large number of cells.

10 Still another object of the invention is the provision of the device for substantial simultaneous injection of large numbers of cells without requiring special operator skills.

15 It is yet a further object of the invention to provide an apparatus for parsimoniously injecting material into the nucleus or into the cytoplasm of each of a plurality of cells.

20 It is a more specific object of the invention to provide an apparatus for injecting a medium to a plurality of cells, including a first structure with a plurality of wells for holding the cells in a regular array, a second structure having a plurality of injection needles spaced in an array corresponding to the regular array of the cells, and a third structure for positioning the first and second structures accurately with respect to one another.

25 Preferably, each of the wells holds only a single cell.

30 The arrays used in the first and second structures may be linear or may be two-dimensional and more specifically rectangular. The wells may be cylindrical, with a counter-sunk conical upper section for guiding the cells thereto. Moreover, the wells may be in the form of

through holes in a plate structure.

Preferably, the first and second structure may be formed on silicon chips utilizing micromachining techniques which are known in the art for fabrication of integrated circuits and the like. The wells may form through holes in the chip forming the first structure and a pressure generating means may be used to retain the cells in the wells by generating a pressure differential. A manifold chamber in sealing arrangement with the surface of the chip opposite to the surface facing the injection needles may be used for this purpose.

The injection needles may be solid or hollow. Hollow needles may include a cavity at a tip portion, or may include a through hole passing through the needles and the chip support therefor. In this embodiment of the invention, a manifold may be provided on the surface of the chip opposing the surface bearing the needles. The manifold is preferably in the form of a chamber for holding the medium to be injected. A pressure generating means may be used to pressurize the manifold in order to inject the medium through the needles into the cells.

The third structure for positioning the first and second structures is preferably arranged to provide perpendicular motion for the two structures relative to one another. Thus, for parallel chips formed to include the wells and the injection needles respectively, the third structure moves the chips perpendicularly towards and away from one another in order to provide contact between the plurality of injection needles and cells contained in each of the wells.

Such a structure may be a tweezer-like arrangement having a pair of arms extending from a flexible hinge.

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An electrically controlled device may be provided for generating the motion, which may include a stepper motor.

Alternatively, at least one of the structures bearing the needles and the wells may have a convex  
5 curvature relative to the other, in order to facilitate contact between the needles and the cells by a rolling motion of that structure relative to the other.

Other objects, features, and advantages of the present invention will become readily apparent to those  
10 skilled in the art from the following description wherein there is shown and described a preferred embodiment of the invention, simply by way of illustration and not of limitation of the best mode (and alternative embodiments) for carrying out the invention. The invention itself is  
15 set forth in the claims appended hereto. As will be realized upon examination of the specification with due reference to the drawings, the present invention is capable of still other, different, embodiments and its several details are capable of modifications in various  
20 obvious aspects, all without departing from the invention which is recited in the claims. Accordingly, the drawings and the descriptions provided herein are to be regarded as illustrative in nature and not as restrictive of the invention.

25 Brief Description of Drawings

In accordance with the above described objects and features of the invention, a preferred embodiment thereof is shown in the accompanying drawings, wherein:

Fig. 1 is a perspective view of an embodiment of  
30 the invention;

Fig. 2 is a detailed section and perspective view of a cell retaining plate which may be used in the

invention;

Fig. 3 is a sectional view of the cell retaining plate of Fig. 2 including a plurality of cells therein;

Fig. 4 is a detailed sectional view of a needle  
5 bearing plate for use in the invention;

Fig. 5 is a partial sectional view of an alternate embodiment of the needle plate of Fig. 4;

Fig. 6 is a partial sectional view of still  
another alternate embodiment of the needle bearing plate  
10 of Fig. 4; and

Fig. 7 is a perspective view of a trough which may be used for temporary filling of a needle bearing plate incorporate needles of the type shown in type 6.

Best Mode for Carrying out the Invention

15 Referring now to Fig. 1, there is illustrated at reference numeral 10 an apparatus according to the invention. As shown therein, a lower plate 12 includes a plurality of cell wells 14. Lower plate 12 retains the cells in wells 14, as would be appreciated upon reference  
20 to Figs. 2 and 3. As illustrated therein, each of the cell wells includes a through hole 16 having a counter-sunk frustoconical upper section 18. When the individual cells to be injected are retained by plate 12, they are preferably seated within the frustoconical sections 18 as  
25 illustrated at Fig. 3. Thus, it is seen that in order to provide an ordered array of cells 20, the through holes 16 are of a smaller diameter than the diameter of the cells. Although the frustoconical upper sections of through holes 16 are shown in the embodiment of Figs. 2  
30 and 3 and provide improved seating of the cells, it will be appreciated by those skilled in the art that the frustoconical sections are not necessary and may be

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omitted.

An upper plate 22, shown in Figs. 1 and 4, includes a plurality of injecting needles disposed in an array matching the array of cell wells on lower plate 12.

5 When lower and upper plates 12 and 22 are properly aligned, a needle 24 is provided opposite each through hole 16. Accordingly, when cells 20 are properly retained by plate 12 in the through holes 16, upper plate 22 may be lowered and, in a single movement, each of  
10 cells 20 will be injected with the injection medium via a corresponding needle 24.

In the preferred embodiment the plates are moved toward one another by a mechanical structure, including a pair of arms 26 and 28, flexibly connected at a  
15 flexible hinge 30. Thus, the hinged structure including hinge 30 and arms 26 and 28, having a general tweezer-like appearance, is used to move the upper and lower plates, which are generally parallel to one another, in a perpendicular direction towards and away from one  
20 another in order to provide in a single movement an injection of a large number of cells.

As seen in Fig. 1, a manifold chamber 32 is disposed below plate 12. The chamber is connected by a pipe 34 to a pressure control device (not shown). A  
25 negative pressure may be provided to the manifold chamber 32 in order to attract each of cells 20 to the through holes 16. Thus, once the cells are seated on the frustoconical sections 18 (or directly on the through holes 16), application of a vacuum retains the cells in  
30 the appropriate locations for injection by needles 24 provided on plate 22.

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In one embodiment of the invention, needles 24 provide through holes in upper plate 22. A second manifold chamber 36 is disposed above the plate 22. A second pipe 38 connects manifold chamber 36 to a fill, inject and rinse control device (not shown). Thus, the medium to be injected may be provided through pipe 38 to manifold chamber 36. Upon application of a positive pressure from the fill, inject and rinse control device, through pipe 38 to manifold chamber 36, the injection medium is provided under pressure by the through holes associated with needles 24 to each of the cells 20. Upon conclusion of the injection process, plates 22 and 12 are separated from one another. Cells 20 may be left on the lower plate 12 in an appropriate maintenance medium, or may be removed into suspension or onto an agar plate, for example, by releasing the vacuum applied to pipe 34 which previously retained the cells in place, and possibly by providing a positive pressure to manifold chamber 32. Upper plate 22 and lower plate 12 may be flushed, cleaned and reused, or may be disposed of.

Plates 12 and 22 may be constructed to have the structure illustrated in Fig. 2 on a microminiature scale, preferably by micromachining a silicon chip utilizing techniques well known in the integrated circuit industry. However, other techniques may be used to fabricate the plates, such as by using etchable glass, diamond grinding, or other techniques. Various techniques are described in an article entitled "Fabrication of Microstructures Using the Liga Process" by W. Ehrfeld, et al., published in the Proceedings of the IEEE Microrobots and Teleoperators Workshop held in Hyannis, Massachusetts, November, 1987 and available from

IEEE under catalogue 87 TH0204-8. It will be appreciated that cell wells 14 would be manufactured to have appropriate diameters for the size of the cells to be injected. Thus, when injecting a number of frog eggs  
5 having diameters of approximately 1 millimeter, the through holes would be provided with diameters of somewhat less than 1 millimeter and thus, on a 1 centimeter by 1 centimeter chip, approximately a 10 by 10 array of cell wells may be provided. Alternatively, when  
10 smaller cells are being injected, such as stem cells having diameters of approximately 10 microns, a 1 centimeter square plate may have an array of 10,000 cell wells arranged in a 100 by 100 array. Thus, with the aid of micromachining, any size array may be manufactured.

15 The inventive structure, unlike the prior art, will work with cells in a suspension. All cell types may be put into suspension, including those which naturally grow on surfaces. Thus, by placing a film of the suspension over the lower plate 12, the suspended cells  
20 are attracted into the wells upon application of suction to pipe 34. As will be appreciated, the structure of Figs. 1-4 provides simultaneous injection of tens of thousands of cells, in a very short period of time, and is highly efficient in utilization of the injection  
25 medium which may be quite rare and/or expensive.

In the illustration of Fig. 3, the nuclei of the various cells are shown as being positioned substantially at the center of the through holes 16. Thus, for a cell population having uniform diameters and nuclei positions,  
30 the inventive structure may be utilized to introduce material into the cell nuclei. The invention thus provides an advantage over the known electroporation

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technique, which does not introduce material into the nucleus. Of course, the present invention could be utilized in combination with electroporation equipment. However, the material is nonetheless provided by  
5 injection into the plural cells.

The manifold chamber 32, sealed to the underside of the chip forming lower plate 12, covers all of the small through holes 16 to permit both positive and negative pressure to be applied thereto. Manifold  
10 chamber 32 is connected by pipe 34 to a pressure control device acting as a source of vacuum or positive pressure. A pump, a syringe or a regulator may be used to provide the varying pressure. The applied pressure, whether positive or negative, may be in the range of 0 to 15 psi.  
15 Typically, the applied pressure differential is sufficient to hold the cells in place for the injection procedure, without permitting the cells to be displaced by bouyancy thereof, by a tendency to sink or due to a stirring operation. However, the pressure differential  
20 is not so high as to rupture the cell wall by extruding the wall into the well holes in the array. Although a wide pressure range (0 to 15 inches of water) is contemplated, a low pressure may be sufficient for retaining frog oocytes, while a greater pressure may be  
25 required for retaining smaller cells in the wells. Moreover, although the cell wells are shown as being circular in shape, it would be clear to those of ordinary skill in the art that other shapes may be used, such as square, elliptical, etc.

30 Upon placing the inventive apparatus 10 in a suspension of cells, or by pouring or flowing such a suspension over the array of recesses, the cells are

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provided between the lower and upper plates. Upon application of suction to manifold chamber 32, a "filtering" process is begun wherein suspension liquid flows through the holes in the chip forming lower plate 12 until, eventually, most or all of the cell wells become filled with cells which cannot pass therethrough but which, instead, nest in the recesses, being retained at least in part by the pressure differential between the two sides of the plate 12. Excessive cells which may accumulate along plate 12 but which are not contained in the cell wells, as well as surplus cell suspension fluid, may be removed by a gentle cross-flushing while the cell wells each continue to contain a cell by the above-noted differential pressure applied thereto.

It is noted that although the inventive structure is illustrated as providing a plurality of needles 24 on upper plate 22, injection may, in fact, proceed by positioning a micropipette in turn over each cell well to provide automatic penetration and injection by a programmed mechanical actuator as previously described. However, while in the prior art the cells are provided in random arrays on a cover slip or petri dish, an arrangement which requires significant expenditures of time to locate the individual cells, the inventive structure provides a regular array of cells. Thus, positioning of the mechanical injection actuator may be programmed into a known numerically controlled machine tool. Accordingly, utilization of a structure incorporating only the lower plate 12 in conjunction with existing mechanical injection actuators provides significant increases in the number of cells which may be injected.

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In order to increase the number of cells being injected still further, the invention incorporates both the lower and upper plates 12 and 22, respectively, as above noted. Such a device may have a cost of five dollars in comparison to a cost of \$80,000 for existing programmed mechanical injection actuators. Moreover, the inventive device may inject 10,000 cells simultaneously while the mechanical actuators are capable of injecting 2,000 cells per hour.

Accordingly, the advantageous structure of Fig. 1 includes significant improvements over presently available devices for injection of large numbers of cells.

The upper plate 22 may be fabricated by etching away material of a silicon chip to leave behind projecting needles having the through holes. However, different injection needle structures may be utilized, as shown in Figs. 5 and 6. Specifically, Fig. 5 illustrates a "poison dart" type of needle shown at 40, wherein a solid sharp projection extends from the surface of upper plate 22 toward a cell well of lower plate 12. In a third embodiment, shown at Fig. 6, the injection needle may be as shown at 42, including a cavity 44 at a tip portion thereof. It will be appreciated that the embodiment of Fig. 5 would be the simplest form to manufacture. Both the embodiments of Fig. 5 and Fig. 6, however, require the use of a shallow trough 46, shown in Fig. 7, for the medium to be injected. To perform the multiple cell injections in accordance with a structure utilizing the embodiments of Figs. 5 or 6, the upper plate 22 is dipped in the trough 46. Capillary action causes the medium to fill the blind hole formed by cavity

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44 in the embodiment of Fig. 6. For the embodiment of Fig. 5, a small amount of the injection material will adhere to the surface of needle 40. Upon removal of trough 46, the upper and lower plates are brought  
5 together, similarly to operation of the embodiment including the upper plate shown in Fig. 4. Each needle penetrates a corresponding cell and the medium then diffuses into the cell.

In still another modification, electroporation  
10 potentials and/or iontophoresis may be used to transfer the injectate into the cells, since the required small volume of injectate has been efficiently positioned for each cell. It will be appreciated that the amount of injectate provided by a needle shown in the embodiment of  
15 Fig. 6 is more strictly and more easily controlled by the size of the cavity 44, thus avoiding waste of precious injectates.

The two dimensional array for holding larger cells, such as frog oocytes, may be formed of a nylon  
20 mesh, or woven net, such as Nylatek. Further, in order to cause the nuclei all to move toward the surface of the cells, a centrifugal force may be applied perpendicularly to the net. When the cells are thus aligned, injection of the nuclei is made simpler.

25 The foregoing description of the preferred embodiment of the invention has been presented for purposes of illustration and description and is not intended to be exhaustive or to limit the invention to the precise forms disclosed, since many modifications and  
30 variations are possible in light of the above teaching. The embodiment was chosen and described in order best to explain the principles of the invention and its practical

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application, thereby to enable others skilled in the art  
best to utilize the invention in various embodiments and  
with various modifications as are suited to the  
particular use contemplated therefor. It is intended  
5 that the scope of the invention be defined by the claims  
appended hereto, when interpreted in accordance with full  
breadth to which they are legally and equitably entitled.

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Claims

1. Apparatus for injection of a medium into a plurality of cells, comprising:  
first means having a plurality of cell wells for  
5 holding a plurality of cells in a regular array;  
second means having a plurality of injection  
needles spaced in an array corresponding to said regular  
array of said cell wells; and  
third means for positioning said first and second  
10 means accurately with respect to one another.
2. Apparatus for injection of a medium into a plurality of cells as recited in claim 1, wherein said regular array is a linear array.
3. Apparatus for injection of a medium into a  
15 plurality of cells as recited in claim 1, wherein each of  
said wells is sized for holding therein only one cell.
4. Apparatus for injection of a medium into a plurality of cells as recited in claim 3, wherein said regular array is a two-dimensional array.
- 20 5. Apparatus for injection of a medium into a plurality of cells as recited in claim 2, wherein said wells are cylindrical.
6. Apparatus for injection of a medium into a plurality of cells as recited in claim 5, wherein said  
25 wells include a counter-sunk conical upper section.
7. Apparatus for injection of a medium into a plurality of cells as recited in claim 1, wherein said first means comprises a chip and said wells comprise  
30 through holes having openings on opposing surfaces of said chip.
8. Apparatus for injection of a medium into a plurality of cells as recited in claim 7, further

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comprising pressure means for providing a pressure differential to retain said cells in said wells.

9. Apparatus for injection of a medium into a plurality of cells as recited in claim 8, wherein said  
5 pressure means comprises a manifold chamber in sealing engagement with one surface of said chip and wherein said second means is positioned on the opposite surface on said chip.

10. Apparatus for injection of a medium into a  
10 plurality of cells as recited in claim 1, wherein said injection needles are substantially solid.

11. Apparatus for injection of a medium into a plurality of cells as recited in claim 1, wherein said injection needles are hollow.

12. Apparatus for injection of a medium into a  
15 plurality of cells as recited in claim 11, wherein each of said injection needles includes a cavity at a tip portion thereof contacting one of said cells.

13. Apparatus for injection of a medium into a  
20 plurality of cells as recited in claim 11, wherein said second means comprises a chip and said injection needles project from one surface thereof to said first means, each of said needles including a through hole passing therethrough and through said chip.

14. Apparatus for injection of a medium into a  
25 plurality of cells as recited in claim 13, further comprising a manifold on a surface of said chip opposite to said one surface, said manifold holding said medium to be injected by said needles.

15. Apparatus for injection of a medium into a  
30 plurality of cells as recited in claim 14, further comprising pressure means for pressurizing said manifold

to inject said medium

16. Apparatus for injection of a medium into a plurality of cells as recited in claim 1, wherein said first and second means each comprises a substantially  
5 planar chip, said planar chips arranged substantially parallel to one another, and said third means comprises means for moving said planar chips towards and away from one another, to provide contact between each injection needle and a cell in each said well.

10 17. Apparatus for injection of a medium into a plurality of cells as recited in claim 16, wherein said means for moving comprises a flexibly hinged structure having a pair of arms in a tweezer-like arrangement.

15 18. Apparatus for injection of a medium into a plurality of cells as recited in claim 17, wherein said means for moving comprises electrically controlled motion generating means.

20 19. Apparatus for injection of a medium into a plurality of cells as recited in claim 18, wherein said electrically controlled motion generating means comprises a stepper motor.

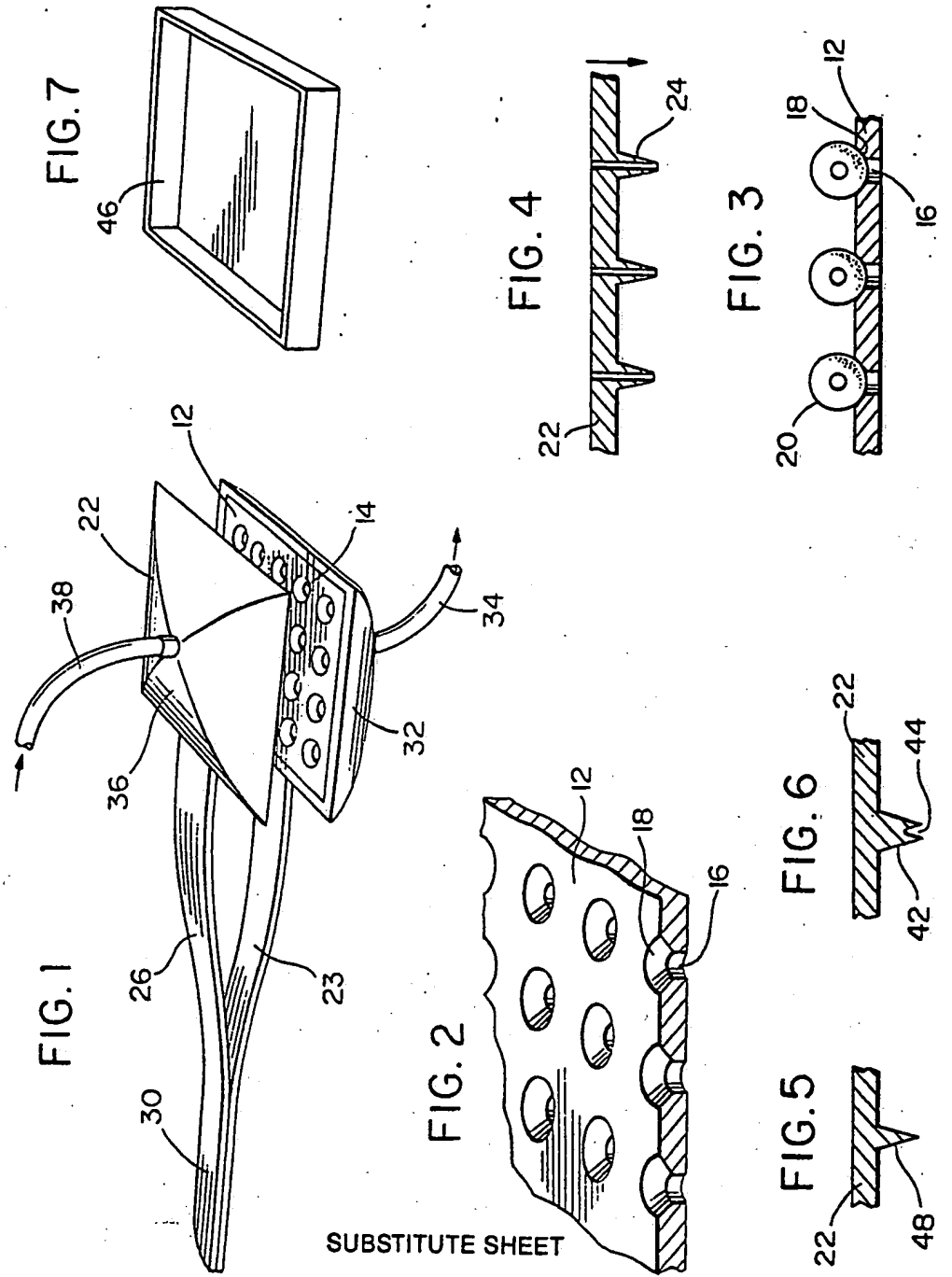
25 20. Apparatus for injection of a medium into a plurality of cells as recited in claim 1, wherein said first and second means each comprises a plate, at least one of said plates having a convex curvature towards the other, thereby to facilitate contact between said needles and cells in said wells by a rolling motion of one plate relative to the other.

30 21. Apparatus for injection of a medium into a plurality of cells, comprising:

first means having a plurality of cell wells for holding a plurality of cells in a regular array;

second means having an injection needle; and  
third means for positioning said first and second  
means accurately with respect to one another for  
injecting the medium into each of the cells in the array.

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# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/05929

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) \*

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61 B 19/00; BC1L 3/00; G01N 1/14

U.S. Cl.: 422/100, 102; 436/63, 165, 180, 809; 435/293, 294, 301; 73/863.31, 864.72

## II. FIELDS SEARCHED

Classification System	Minimum Documentation Searched <sup>7</sup>
US. Cl.	422/100, 102; 436/63, 165, 180, 809; 435/293, 294, 301; 73/863.31, 864.72

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched \*

## III. DOCUMENTS CONSIDERED TO BE RELEVANT \*

Category *	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X Y	US. A. 4,158,035 (HAASE ET AL) 12 June 1979. See entire document.	1-5,11-12 &20-21 18-19
X	US. A. 4,162,896 (HOSLI) 31 July 1979. See entire document.	1-5,10-12& 20-21
X Y	US. A. 4,276,048 (LEABACK) 30 June 1981. See entire document.	1-5,11-12 &20-21 18-19
X Y	US. A. 4,461,328 (KENNEY) 24 July 1984. See entire document.	1-5,11-12 &20-21 18-19
X Y	US. A. 4,478,094 (SALOMAA ET AL) 23 October 1984. See entire document.	1-5,11-12 &20-21 18-19
X	US. A. 4,599,315 (TERASAKI ET AL) 08 July 1986. See entire document.	1-6,10-12 &20-21
X	US. A. 4,695,547 (HILLIARD ET AL) 22 September 1987. See entire document.	1-5,10& 20-21

### \* Special categories of cited documents: \*

- "A" document defining the general state of the art which is not considered to be of particular relevance
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

23 January 1991

International Searching Authority

ISA/US

Date of Mailing of this International Search Report

04 MAR 1991

Signature of Authorized Officer

L. Kummert

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	US, A, 4,619,899 (NIKI TIN ET AL) 28 October 1986.	
A	US, A, 4,664,097 (MCGRATH ET AL) 12 May 1987.	
Y	IEEE Proceedings, issued November 1987 Micro Robots and Teleoperators Workshop, "Fabrication of Microstructures using the LIGA Process", Ehrfeld et al., See entire document.	7-9&13-16